REMARKS

Summary of the Invention

The invention provides methods of transplanting retinal cells by implanting isolated retinal stem cells from the retina of a mammal and/or implanting retinal cells differentiated from the retinal stem cells, into the retina of an individual.

Summary of the Office Action

Claims 5-8 are pending and are rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement.

Support for the Amendments

Support for the amendments to claims 6 and 7 is found on page 1, lines 15-17, page 2, lines 9-11, and page 12, lines 19-21. Support for claim 9 is found on page 11, line 22, through page 12, line 2, and page 20, line 21, through page 23, line 11. No new matter is added by the amendment.

Informalities

As requested by the Examiner, Applicants provide herewith a clean copy of the figures referenced in the Declaration of Dr. Tropepe, filed April 11, 2002.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 5-8 are rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement.

The Examiner states that "the instant claims are drawn to methods of treating individuals (humans) with a degenerative disease, disorder or abnormal state of the retina or eye...comprising implanting retinal stem cells or retinal cells differentiated from retinal stem cells." See page 3, lines 4-7, of paper no. 15. Furthermore, the Examiner states that to practice the claimed invention:

> the skilled artisan would have to overcome the immune response problem that is well documented in the art, then the artisan would need to establish appropriate transplantation sites, appropriate cell numbers to [use] for each condition found recited in the claims. In addition, the skilled artisan would need to develop appropriate assays to determine which protocols were efficacious. See page 6, lines 20-24, of paper no. 15.

Finally, the Examiner states that:

> it is not clear based upon the art of record that the animal model system used by [the inventors] is art recognized as predictive of success in treating a human animal for any disease, disorder or abnormal physical state of the eye (e.g. those listed in claim 7). See page 8, lines 11-13, of paper no. 15.

Applicants respectfully disagree.

During a telephone interview between the undersigned and Examiners Yucel and Leffers on January 13, 2003, the Examiners stated that Applicants have satisfied the "how to make the invention" provision of the enablement requirement, but have failed to satisfy the "how to use the invention" provision of the enablement requirement because the only use provided in the specification for the transplantation of retinal cells is the treatment of diseases, disorders, or abnormal physical states of the retina of the eye. The Examiners stated that this use is not enabled because there is no teaching in the specification for overcoming the immune response and there is no proof that the animal model system used by the inventors is art recognized as

predictive of success in treating a human animal for any disease, disorder, or abnormal physical state of the retina of the eye.

The Examiner raises four distinct issues with respect to the "how to use" provision of the enablement requirement: (i) the knowledge of one skilled in the art with regard to the transplantation of retinal cells at the time the application was filed; (ii) the likelihood that the animal model system used by the inventors is art recognized as predictive of success in a human animal; (iii) the avoidance of an adverse immune response; and (iv) the success of treatment of any disease, disorder, or abnormal physical state of the retina of the eye (e.g., those conditions listed in claim 7) using the method of present claim 5. Applicants address these issues below.

As an initial matter, Applicants direct the Examiner to the amendments to claims 6 and 7, in which Applicants have amended the claims to specify that the individual is suffering from a retinal disease or disorder, or an abnormal physical state of the retina of the eye. The Examiner expressed concern during the telephone interview of January 13, 2003 that claim 6 did not specify that the disease or disorder, or abnormal physical state of the eye was a result of damage to or loss of retinal cells, which condition could be ameliorated by providing retinal cells. Accordingly, claim 6 has been amended to address this issue. Claim 7 has also been amended to specify that the condition of blindness is caused by damage or disease to the retina of the eye, thereby clarifying that the condition of blindness is not neurological in nature. Applicants submit that the other conditions listed in claim 7 are clearly understood by one skilled in the art to be associated with damage or disease to retinal cells. Accordingly, the claims are now understood to specify that the individual is suffering from a condition that is strictly related to the retina, and therefore, it is plausible that the presently claimed method of transplanting retinal stem cells,

which can differentiate into the various types of retinal-derived cells, is related to the amelioration of a condition associated with the loss of retinal cells.

The Transplantation Methods were Art-Recognized Prior to Applicants Filing Date

With respect to the first issue discussed above, Applicants direct the Examiner to the Declaration of Dr. Tropepe, filed with the Reply to Office Action on April 11, 2002 (hereinafter referred to as "the Declaration of Dr. Tropepe") and the Second Declaration of Dr. Tropepe filed herewith (hereinafter "the Second Declaration of Dr. Tropepe). Dr. Tropepe - an expert in his field - states that the "materials and methods employed were known to those skilled in the art of cellular transplantation at the time the application was filed or are provided by the instant specification..." See paragraph 5 of the Declaration of Dr. Tropepe and paragraph 5 of the Second Declaration of Dr. Tropepe. These statements confirm that the transplantation methods used to practice the claimed method were known in the art prior to filing the present application. In addition, Applicants also provide evidence that the skilled artisan clearly recognized how to transplant cells into the retina of an mammal at the time the application was filed (see, e.g., Muller-Jensen et al., Mod. Probl. Ophthamol. 15:228-234, 1975; submitted previously; Whiteley et al., Experimental Neurology 140:100-104, 1996; provided herewith; and He et al., Graefes Arch. Clin. Exp. Ophthamol. 231:737-742, 1993; provided herewith). Accordingly, the transplantation of cells into the retina of an individual (via subretinal or intravitreal injection) was art-known prior to the filing date of the present application and does not represent the inventive contribution of the present invention.

The Mouse Model of Retinal Stem Cell Transplantation is Predictive of Success in Humans

The previously provided Declaration of Dr. Tropepe provided data that demonstrated that retinal stem cells isolated from adult mice carrying the green fluorescent protein (GFP) transgene could be transplanted into the vitreous of the eye of recipient mice without the occurrence of an adverse immune response (see paragraphs 5-8 of the Declaration of Dr. Tropepe). The transplanted cells were detected within several layers of the multi-layered retina indicating that they had survived and migrated to a proper position in the eye. The retinal stem cells were also observed to differentiate into at least two different cell types (i.e., Müller glial cells and rod photoreceptors). Based on these results, Applicants conclude that transplanted retinal stem cells can be successfully used to replenish retinal stem cells that have been lost due to damage or disease and that these cells can likely restore lost retinal function. The Examiner questions whether this result is predictive of success in humans with respect to both transplantation and the restoration of retinal function. In response, Applicants again refer the Examiner to the Second Declaration of Dr. Tropepe, which states that the success Applicants observed in the mouse model is considered predictive of success in humans with respect to both the transplantation of retinal stem cells and the restoration of retinal function due to the replenishment of lost retinal cells, and would be so recognized by one skilled in the art of retinal transplantation (see paragraphs 6 and 8 of the Second Declaration of Dr. Tropepe). Accordingly, the successful transplantation of retinal cells, and their differentiation *in vivo*, while demonstrated in the mouse system, would be accepted by one skilled in the art as predictive of the result obtained if the transplantation of retinal stem cells was performed in a human system.

Four Weeks is Sufficient Time for Evaluating the Presence or Absence of an Immune Response

In response to the Examiner's third concern regarding the potential for an adverse immune response, Applicants refer the Examiner to the Declaration of Dr. Tropepe, which states that the mice were examined after a four week survival period and "[t]he recipient mice of the experiments...were healthy and active and microscopic examination of recipient retinae revealed no abnormal retinal development or adverse immune response." See paragraphs 5 and 8 of the Declaration of Dr. Tropepe, and paragraph 7 of the Second Declaration of Dr. Tropepe. Accordingly, after four weeks of observation the transplanted retinal cells did not elicit an immune response. As has been noted by Applicants and in the art, this is because the retina is an immune-privileged site.

Applicants submit that four weeks is considered a sufficient time period for evaluating the immune response to transplantation of retinal cells in the eye by those skilled in the art. In support of this conclusion, Applicants point to the references cited by the Examiner, i.e., Grisanti et al., Enzmann et al., and Crafoord et al., which describe the lack of an immune response due to the transplantation of retinal cells after 12 days (see, e.g., page 1622, of Grisanti et al.), 6 weeks (see, e.g., page 180 of Enzmann), and 1, 3, or 6 months (see, e.g., page 249, col. 1, of Crafoord et al.), respectively. All of the references state that no immune response by inflammatory cells (e.g., lymphocytes and plasma cells; i.e., classical acute graft rejection) was detected at any of the time during the examination period; a clear indication that these time periods were each considered sufficient to detect such a response. Crafoord et al. states that at 6 months an

immunological response consisting of large macrophages was observed, but that classical acute graft rejection was not present. Crafoord et al. suggests that the immunological response may have been modified by ACAID or a similar mechanism, thereby allowing grafts "to survive in the subretinal space much longer than observed in immunologically non-privileged sites..." See page 253, col. 1, of Crafoord et al. Gristanti et al. discloses that, by contrast, after only 12 days the transplantation of RPE cells into the subconjunctival space, a non-immune privileged site that is actively monitored by the immune system, resulted in a clear and significant immune reaction that involves inflammatory cells (i.e., lymphoid cells, polymorphonuclear cells, and mononuclear cells; i.e., the classical acute graft rejection). Therefore, Grisanti et al. clearly demonstrates that transplantation of retinal cells into a non-privileged site (i.e., a site other than the retina) will trigger an acute graft rejection within the time frame used in the experiments described in the Tropepe Declaration of April 11, 2002. This acute graft rejection is clearly not observed by the inventors in the present case.

In addition, Applicants direct the Examiner to He et al. (*supra*), which was published prior to the filing date of the present application. He et al. states that human RPE cells transplanted into the eye of a rabbit did not elicit an immune response involving lymphocytes when observed from 1 week to 3 months. Similar to the observations of Crafoord et al., the authors noted the presence of macrophages, but not lymphocytes, at three months. The authors conclude that the subretina is relatively immuno-privileged and that graft rejection is not an insurmountable problems and could likely be eliminated by the use of immunosuppressants (see pages 740-741 of He et al.).

Finally, Applicants direct the Examiner to the post-filing art of Siegel et al., provided

previously, which also states that the immune response to transplanted retinal cells was investigated after 1 month with no evidence of immune rejection (see, e.g., page 562, of Siegel et al.). Based on these five references, Applicants contend that the region of the retina targeted with retinal stem cells and differentiated retinal stem cells is immuno-protected and thus amenable to transplantation. Nonetheless, the Declaration of Dr. Tropepe provides factual consideration of this conclusion.

Furthermore, Applicants submit that both short-term and long-term immunosuppression is a common intervention for all forms of transplantation (e.g. lung, liver, kidney) and in many cases may be accomplished without serious side effects. The extent of immunosuppression for patients with only tens of thousands of retinal stem cells and their progeny transplanted is likely to be minimal (at least compared to those patients whole receiving vital organs) and the concern for side effects may be negligible. Therefore, even in the absence of any demonstration that a long term immunological response might be expected (i.e., greater than 4 weeks), such response could be mitigated using immunosuppression, and such therapeutic protocols are well within the purview of the skilled artisan (see, e.g., He et al., *supra*).

A Skilled Artisan would Recognize that the Present Invention is Useful for the Treatment of Retinal Cell-Associated Diseases or Disorders

Finally, the Examiner requests data demonstrating that the method will treat any of the diseases, disorders, or abnormal physical states of the retina of the eye listed in claim 7 for any period of time. To address this concern, Applicants respond by again directing the Examiner to the Declaration of Dr. Tropepe. Applicants submit that the data presented in the Declaration not

only demonstrates the successful transplantation of retinal stem cells, but also the survival, differentiation, and proper positioning of the transplanted retinal stem cells. The Declaration states that:

> Extensive integration of GFP+ donor cells within the host retina after a 4-week survival period was observed. Donor cells were detected within several layers of the multi-layered retina. For instance, GFP+ cells were observed in the area of the inner nuclear layer juxtaposed with the inner and outer plexiform layers. Expression of the Müller glial cell marker 10E4 (which labels glial cells extending throughout the entire extent of the retina) appears to correlate with a small fraction of the donor cells in the inner nuclear layer. The vast majority of donor cells, however, appeared to migrate through the inner cell layers of the retina (ganglion cell layer, and inner nuclear layer) and settle in the outer nuclear layer of the retina (photoreceptor cell layer) (Figure 1, a copy of which is attached as Appendix B). The GFP+ cells in the outer nuclear layer resembled rod photoreceptors in morphology and expressed several markers specific for mature rods. For instance, the rod marker Rho4D2 corresponded to the position of the outer segment of the rod cells and was co-expressed with GFP in these outer segments (see Figure 1, Panel B). Other rod markers (B630, 7H2, D2P4) were similarly co-expressed in GFP+ cells in this region. In some instances, the extent of integration of these cells in the photoreceptor layer was significant, yet layer morphology was largely undisrupted. Thus, these data suggest that a large number of retinal stem cell-derived cells differentiate into mature rod photoreceptors with normal morphological and biochemical properties after transplantation. (See paragraph 6, of the Declaration of Dr. Tropepe.)

Based on this data, Applicants submit that the retinal stem cells are capable of replenishing different types of diseased or damaged retinal cells by migrating to the proper position in the eye and differentiating into these different cell types. This result suggests that successful treatment of an individual with a disease or disorder that is characterized by a loss of functional retinal cells can be achieved by transplanting retinal stem cells into the retina of that individual.

In support of this conclusion, Applicants again direct the Examiner to He et al. (*supra*), which states that the transplantation of human RPE cells into the retina of a rabbit results in restored function of the RPE layer as evidenced by "the presence of phagosomes and phagocytosed outer segments in the transplanted cells." See the abstract of He et al. He et al. also states that "[o]ur results in conjunction with those of others...suggest that... [i]mmunosuppressive therapy with drugs such as cyclosporine might well eliminate...[any] deleterious response." See page 741 of He et al.; emphasis added. He et al. concludes that their results "suggest an eventual role for RPE transplantation in the treatment of human diseases such as retinitis pigmentosa and age-related macular degeneration." See page 740 of He et al.

Several other post-filing publications also support Applicants' claim that successful recovery of retinal function occurs by providing a source of new, undamaged retinal cells. Applicants first refer the Examiner to the post-filing art of Sauvé et al. (Experimental Neurology 152:243-250, 1998; provided herewith), in which retinal pigment epithelial (RPE) cells are transplanted into a rat model of progressive retinal degeneration. Sauvé et al. states that the dystrophic RCS rat model may provide a useful model for the treatment of retinitis pigmentosa and age-related macular degeneration (see page 243, col. 2). Sauvé et al. demonstrates that the transplantation of RPE cells into either the subretinal space or the intravitreal space results in RPE cell survival and rescue of photoreceptor function, and concludes by stating that "transplantation can have a direct and effective influence on the deterioration of visual responsiveness..." See page 246, col. 2, and page 248, col. 2.

Applicants also provide Whiteley et al. (Experimental Neurology 140:100-104m 1996), which discloses that subretinal transplantation of RPE cells into dystrophic RCS rats results in

the rescue of photoreceptors. See pages 101-102 of Whiteley et al. Whiteley et al. concludes by stating that "[t]hese results confirm...the efficacy of RPE cell grafts in rescuing photoreceptors...[and] provide evidence that the rescued cells affect a minimum of visual function..." See page 101 of Whiteley et al.

In total, these three publications support Applicants' contention that the art recognizes the therapeutic/restorative benefits associated with the successful transplantation of RPE cells. This benefit would clearly extend to the transplantation of retinal stem cells, as is taught by the present application, because these cells are capable of differentiating into the different cell types of the retina (including, e.g., rod photoreceptor cells and Müller glial cells; see, e.g., paragraph 6 of the Declaration of Dr. Tropepe).

Summary

In short, Applicants submit that, with respect to all of the issues discussed above, the two Declarations of Dr. Tropepe provide compelling data to support Applicants' position that the claimed invention is enabled. Dr. Tropepe has not only provided an expert opinion as to the successful practice of the presently claimed method, he has also supported that position with clear facts, data, and reasoning. Such evidence and reasoning cannot be dismissed in the absence of compelling evidence to the contrary, and such evidence is not provided in this case. Therefore, in view of the above remarks, Applicants submit that the present specification is more than sufficient to teach those skilled in the art both "how to make" and "how to use" the claimed invention. Accordingly, the rejection of claims 5-8 under 35 U.S.C. § 112, first paragraph, for lack of enablement should be withdrawn.

CONCLUSION

Applicants submit that the claims are in condition for allowance and such action is respectfully requested. Enclosed is a Petition to extend the period for replying for three months, to and including January 16, 2003. Also enclosed is a check in the amount of $465.00 as required by 37 C.F.R. §1.17(a).

Applicants note that the Office Action was mailed to the incorrect address. Effective immediately, please address all communication in this application to:

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If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: January 16, 2003

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Version with markings to show changes made

In the Claims:

A marked-up version of claims 6 and 7 is presented below.

6. (Amended) The method of claim 5, wherein said mammal is a human, and said individual is a human, and said individual is suffering from a retinal disease[,] or disorder, or an abnormal physical state of the retina of the eye.

7. (Amended) The method of claim 6, wherein said retinal disease[,] or disorder, or abnormal physical state of the retina of the eye is selected from the group consisting of blindness due to disease or damage to the retina of the eye, cytomegalovirus retinitis, uveitis, glaucoma, macular degeneration, retinitis pigmentosa, retinal degeneration, retinal detachment, and cancers of the retina.

Pending Claims

5. A method of transplanting retinal cells comprising implanting (i) isolated retinal stem cells from the retina of a mammal and/or (ii) retinal cells differentiated from said retinal stem cells, into the retina of an individual.

6. (Amended) The method of claim 5, wherein said mammal is a human, and said individual is a human, and said individual is suffering from a retinal disease or disorder, or an abnormal physical state of the retina of the eye.

7. (Amended) The method of claim 6, wherein said retinal disease or disorder, or abnormal physical state of the retina of the eye is selected from the group consisting of blindness due to disease or damage to the retina of the eye, cytomegalovirus retinitis, uveitis, glaucoma, macular degeneration, retinitis pigmentosa, retinal degeneration, retinal detachment, and cancers of the retina.

8. The method of claim 5, wherein said retinal cells are differentiated from said retinal stem cells *in vitro*.

9. (New) The method of claim 5, wherein the retinal stem cells comprise cells isolated from a retinal pigment epithelial layer of the retina and/or cells derived therefrom.